

Application No. 09/908,943
Amendment dated November 16, 2006
Reply to Office Action of May 18, 2006

Docket No.: 29915/00281A.US

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AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the applications.

Listing of Claims:

Claims 1-103 (canceled)

104. (currently amended) The method of claim [[102]] 110, wherein the peptide comprises a sequence of amino acids defined by the formula P₂P₁-P₁P₂P₃, wherein
~~P₂ comprises an amino acid selected from the group consisting of N, S, and D;~~
~~P₁ comprises an amino acid selected from the group consisting of Y, F and L;~~
~~P₁ comprises an amino acid selected from the group consisting of E, A, and D;~~
~~P₂ comprises an amino acid selected from the group consisting of A and V;~~
P₃ comprises an amino acid selected from the group consisting of E, G, F, H, cysteic acid and S.

105. (currently amended) The method of claim [[102]] 110, wherein the peptide comprises a sequence of amino acids defined by the formula P₂P₁-P₁P₂P₃, wherein
~~P₂ comprises an amino acid selected from the group consisting of S, N, F, and K;~~
~~P₁ comprises an amino acid selected from the group consisting of F, L, Y, and M;~~
~~P₁ comprises an amino acid selected from the group consisting of E, D, and A;~~
~~P₂ comprises an amino acid selected from the group consisting of A and V;~~
P₃ is E.

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106. (currently amended) The method of claim [[102]] 104, wherein the peptide comprises a sequence of amino acids defined by the formula P₃P₂P₁-P₁P₂P₃, wherein P₃ is an amino acid selected from the group consisting of A, V, I, S, H, Y, T and F.

107. (previously presented) The method of claim 106, wherein P₃ comprises an amino acid selected from the group consisting of I or V.

108. (previously presented) The method of claim 106, wherein the peptide comprises a sequence of amino acids defined by the formula P₄P₃P₂P₁-P₁P₂P₃, wherein P₄ is an amino acid selected from the group consisting of E, G, I, D, T, cysteic acid and S.

109. (previously presented) The method of claim 108, wherein the peptide comprises a sequence of amino acids defined by the formula P₄P₃P₂P₁-P₁P₂P₃P₄, wherein P₄ is an amino acid selected from the group consisting of F, W, G, A, H, P, G, N, S, and E.

110. (currently amended) The-A method for assaying for modulators of β-secretase activity, comprising:

(a) contacting a polypeptide with β-secretase APP processing activity with a substrate, both in the presence and in the absence of a putative modulator compound;

wherein said substrate comprises a peptide having an amino acid sequence of at least 6 amino acids, said amino acid sequence including four amino acids defined by formula P₂P₁-P₁P₂, of claim 102, wherein the amino acids at positions P₂, P₁, P₁, P₂ comprise S, Y, E and V, respectively,

(b) measuring cleavage of the substrate peptide in the presence and in the absence of the putative modulator compound; and

(c) identifying modulators of β-secretase activity from a difference in substrate cleavage in the presence versus in the absence of the putative modulator compound, wherein a modulator that is a β-secretase antagonist reduces such cleavage and a modulator that is a β- secretase agonist increases such cleavage.

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111. (previously presented) The method of claim 110, wherein said peptide comprises the amino acid sequence SEISY-EVEFR (SEQ ID NO: 152).

112. (previously presented) The method of claim 110, wherein said peptide comprises the amino acid sequence SEISY-EVEFRWK (SEQ ID NO: 190).

113. (previously presented) The method of claim 110, wherein said peptide comprises the amino acid sequence GLTNKTEEISEISY-EVEFRWK (SEQ ID NO: 191).

114. (currently amended) The method of claim 110, wherein said peptide comprises the amino acid sequence SEVSY-EVEFR (SEQ ID NO: 141).

115. (previously presented) The method of claim 110, wherein said peptide comprises the amino acid sequence KTEEISEVSY-EVEFR (SEQ ID NO: 147).

116. (previously presented) The method of claim 115, wherein said peptide comprises the amino acid sequence TRPGSGLTNKTEEISEVSY-EVEFR (SEQ ID NO: 145).

117. (canceled)

118. (currently amended) The method of claim [[102]] 110, wherein said substrate comprises an amyloid precursor protein (APP) amino acid sequence with a modified β -secretase processing site defined by said formula P₂P₁-P₁-P₂.

119. (currently amended) The method of any one of claims 102-117 110-116 or 118, wherein said peptide comprises an amino acid sequence having up to 50 amino acids.

120. (currently amended) The method of any one of claims 102-118 110-116 or 118 wherein the peptide further comprises a first label.

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121. (previously presented) The method of claim 120 wherein the peptide further comprises a second label.

122. (currently amended) The method of any one of claims ~~102-117~~ 110-
116 or 118 wherein the peptide further comprises a detectable label and a quenching moiety, wherein cleavage of the peptide between P₁ and P_{1'} separates the quenching moiety from the label to permit detection of the label.

123. (currently amended) The method of claim ~~103-108~~, wherein said cysteic acid comprises a covalently attached label.

124. (currently amended) The method of any one of claims ~~102-118~~ 110-
116 or 118, wherein the rate of cleavage of said peptide by said human aspartyl protease is greater than the rate of cleavage of a polypeptide comprising the human APP β-secretase cleavage sequence: SEVKMDAEFR (SEQ ID NO: 20).

125. (currently amended) The method of any one of claims ~~102-118~~ 110-
116 or 118, wherein the rate of cleavage of said peptide by said human aspartyl protease is greater than the rate of cleavage of a polypeptide comprising the human APP Swedish KM→NL mutation, β-secretase cleavage sequence SEVNLDAEFR (SEQ ID NO: 19).

126. (currently amended) The method of any one of claims ~~102-118~~ 110-
116 or 118, wherein the polypeptide with β-secretase APP processing activity comprises an amino acid sequence selected from the group consisting of

(a) the amino acid sequence of SEQ ID NO: 2,

(b) a fragment of the amino acid sequence of SEQ ID NO: 2 that retains β-secretase APP processing activity, wherein said fragment includes the aspartyl protease active site tripeptides DTG and DSG,

(c) an amino acid sequence that is at least 95% identical to (a) or (b),

wherein the polypeptide includes the aspartyl protease active site tripeptides DTG and DSG and exhibits β-secretase APP processing activity;

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- (d) the amino acid sequence SEQ ID NO: 4;
- (e) a fragment of the amino acid sequence of SEQ ID NO: 4 that retains β-secretase APP processing activity, wherein said fragment includes the aspartyl protease active site tripeptides DTG and DSG, and
- (f) an amino acid sequence that is at least 95% identical to (d) or (e), wherein said fragment includes the aspartyl protease active site tripeptides DTG and DSG and exhibits β-secretase APP processing activity.

127. (currently amended) The method of any one of claims ~~102-118~~ 110-116 or 118, wherein the polypeptide with β-secretase APP processing activity comprises an amino acid sequence selected from the group consisting of

- (a) the amino acid sequence of SEQ ID NO: 2; and
- (b) a fragment of the amino acid sequence of SEQ ID NO: 2 that retains β-secretase APP processing activity, wherein said fragment includes the aspartyl protease active site tripeptides DTG and DSG.

128. (previously presented) A method according to claim 126, wherein the polypeptide with β-secretase APP processing activity comprises a polypeptide purified and isolated from a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the polypeptide.

129. (previously presented) A method according to claim 118,
wherein the substrate is expressed in a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the substrate,
wherein the cell expresses the polypeptide with β-secretase APP processing activity;
wherein the contacting comprises growing the cell in the presence and absence of the test agent, and
wherein the measuring step comprises measuring APP processing activity of the cell.

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130. (previously presented) A method according to claim 129, wherein the contacting comprises administering the test agent to a transgenic non-human mammal that comprises the cell.

131. (currently amended) A method according to claim [[102]] 110, wherein the polypeptide is encoded by a polynucleotide comprising the nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO; 3,
- (b) a nucleotide sequence that hybridizes under the following stringent hybridization conditions to the complement of SEQ ID NO: 1 or 3:
 - (1) hybridization at 42°C in a hybridization buffer comprising 6x SSC and 0.1% SDS, and
 - (2) washing at 65°C in a wash solution comprising 1x SSC and 0.1% SDS;

wherein said nucleotide sequence encodes a polypeptide that exhibits β -secretase APP processing activity.